Report on the Genetics of Coho Salmon (*Oncorhynchus kisutch*) held at Warm Springs (Don Clausen) Hatchery for Recovery Efforts in the Russian River

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December 2003

Introduction

Coho salmon (*Oncoryhnchus kisutch*) populations in California have suffered severe population declines in the last several decades, with many sites that have historical records of the species no longer supporting breeding populations (Spence et al. 2001). The situation is particularly dire in the Central California Coast ESU, which extends from south of the Mattole River to Santa Cruz county (Weitkamp et al. 1995), where only a handful of sizeable, persistent populations remain. The Russian River is the largest watershed in this ESU, but has suffered near extinction of its coho salmon population in recent decades. Efforts to reverse declines in Russian River coho salmon have included hatchery supplementation and habitat restoration. Unfortunately, these efforts have been largely unsuccessful and coho salmon are found consistently, albeit at very low abundance, only in Green Valley Creek. Small numbers of juvenile coho salmon have also been observed in several other Russian River tributaries.

In 2001, state and federal agencies, public interest groups and other stakeholders came together to initiate an *ex situ* recovery program for the Russian River coho salmon population. This multi-year recovery program involves the capture of juvenile fish in areas that are at high risk of drying up. These fish are transferred to the captive facility at Warm Springs (Don Clausen) Hatchery (WSH) and held for the remainder of their lifecycle. The intention is to breed them at reproductive maturity and release the offspring into Russian River tributaries that are currently unoccupied by the species.

At the time of program initiation, very little was known about the genetics of coho salmon in California. Several issues of importance to such a program can be addressed using genetic data. First, a basic assessment of genetic variability can provide insight into risks due to inbreeding depression and lack of evolutionary potential. Because of the extremely low number of coho salmon found in the Russian River basin, concerns about inbreeding depression, due to a limited number of founders in the population, were paramount in designing the program. A related question involves the elucidation of the ancestral relationships of Central California Coast ESU populations. One potential strategy for mitigating small population effects, such as inbreeding, in such a recovery program is an infusion of genes from other populations to increase variability and effective population size. However, crossing genetically distinct lineages can run the risk of outbreeding depression, or reduced fitness in the descendent generations, and raises the question of which stock might be an appropriate donor. The Lagunitas/Olema Creek watershed harbors the most geographically proximate, persistent, population of coho salmon and is a good *a priori* donor candidate, should such supplementation be appropriate. Finally, genetic data can provide a breeding matrix that minimizes inbreeding and maximizes effective population size, by avoiding matings between closely related individuals and maximizing the conservation of rare variants. This is especially important in captive breeding programs, because the inbreeding avoidance mechanisms present in most natural populations cannot be expressed.

Of all the genetic markers used for population biology, microsatellite genes (loci) are the most common and powerful. Microsatellites are non-protein-coding genes found in abundance in all eukaryotic genomes. They consist of small segments of DNA with a short sequence, typically 2-6 nucleotides in length, that is repeated many times in tandem. Most are selectively neutral and highly variable in the number of repeats. This size variation is extremely informative statistically and this, coupled with the ability to derive

data from small, non-lethally obtained tissue samples, is the main reason that they have become one of the most important tools in modern biology, with applications including DNA fingerprinting, gene mapping, ecology, conservation and fisheries management.

In this report, we describe the genetic analysis of fish captured for the first year of this *ex situ* recovery program. We use data from 18 microsatellite loci to describe variation in fish collected in the Russian River tributaries and in Lagunitas/Olema Creek. We also assess the relationship between the fish collected in the two basins and evaluate it in the context of relationships between other coho salmon populations in California. We then use the genetic data to construct a breeding matrix for the Russian River fish that will minimize inbreeding and maximize effective population size in the captive breeding portion of the project. Finally, we draw upon these data and other aspects of the species' biology to make recommendations about several aspects of program management.

Methods

In July of 2002, tissue samples from the 308 fish being held at Warm Springs Hatchery were transferred to the Santa Cruz Laboratory (SCL) for genetic analysis. This includes fish from three tributaries of the Russian River: Green Valley (N=189), Mark West (N=4) and Mayacama (N=1) Creeks, as well as fish from Lagunitas/Olema Creek (N=114) in Marin County. All fish were captured in the summer of 2001 as juveniles and should become reproductively mature in the winter of 2003-04.

DNA was extracted from all tissue samples using a BioRobot 3000 (Qiagen Inc.) and the filter-based DNeasy system (Qiagen Inc.). The use of automation reduces the chance for sample mix-up. Recoverable DNA was obtained from all samples. All DNA extractions were subjected to PCR amplification for 18 microsatellite loci. (Table 1).

Table 1: Microsatellite loci used in current study

Locus Name	**Reference**
Omm 1058	Rexroad et al. 2002
Omm 1080	Rexroad et al. 2002
Omm 1116	Rexroad et al. 2002
Ots G3	Williamson et al. 2002
OtsG68	Williamson et al. 2002
Ots G78b	Williamson et al. 2002
Ots G83b	Williamson et al. 2002
Ots G422b	Williamson et al. 2002
Ots 1b	Banks et al. 1999
Ots 103	Beacham et al. 1998
Oki 1	Smith et al. 1998
Oki13	Smith et al. 1998
Ssa14	McConnell et al. 1995
Ssa 85	O'Reilly et al. 1996
One13	Scribner et al. 1996
One11b	Scribner et al. 1996
Ocl8	Condrey and Bentzen 1998
p53	de Fromentel et al. 1992

These 18 microsatellite loci have been described previously and extensively tested by our and other laboratories. They are the same set of genes being used for larger studies of population structure in coho salmon by the Santa Cruz Laboratory. The 18 loci examined here were chosen to represent different marker types (e.g. di- and tetra-nucleotide repeats), different levels of variation (i.e. known numbers of alleles in other coho salmon populations) and different species of origin (e.g. Ssa14: *Salmo salar* and Oki13: *O. kisutch*). This was done to minimize ascertainment biases or other biases due to characteristics specific to one species or type of marker.

PCR was carried out in 15μl volume reactions in 96 well microplates. All DNA was transferred from extraction plates to PCR plates by robot to eliminate potential sample mix-ups. All PCR reactions used AmpliTaq (Applied Biosystems Inc.) polymerase and were performed according to manufacturer's recommendations with a standard reaction protocol. Genotype determination was then carried out through electrophoresis in 5% acrylamide gels on an ABI 377 Automated DNA Sequencer (Applied Biosystems, Inc.). Fragment size analysis was performed using the GeneScan and Genotyper software packages. Two people performed all fragment size calls independently, with any discrepancies resolved with both callers present. If there was continuing ambiguity or disagreement, no data were recorded. This situation involved a very small number of individual genotypes. Once data were finalized, we used a variety of standard software packages to perform population genetic analyses.

Results

Measures of genetic variation

Genetic diversity can be measured in many ways. One is heterozygosity, which is simply the observed or expected probability that any individual gene carries two different variants or, conversely, the percentage of genes in an individual or population that are found in heterozygous form. Heterozygosity is relatively high in both populations, with expected heterozygosity significantly higher in Lagunitas/Olema than in Green Valley, and observed heterozygosity non-significantly higher in Green Valley (Table 2). The difference between observed and expected heterozygosity is large for Green Valley, but trivial for Lagunitas/Olema. Higher observed vs. expected heterozygosity is a hallmark of recently bottlenecked populations and this transient heterozygosity excess is the basis for one of most common bottleneck detection tests (Cornuet and Luikart 1996). Indeed, this test provides evidence for a recent bottleneck in Green Valley, as does the M-ratio test (Garza and Williamson 2001). The results of these tests are non-significant for the Lagunitas/Olema population (results not shown). Both of these bottleneck tests rely on the relative insensitivity of many measures of genetic diversity, such as heterozygosity, to reductions in population size when compared with a direct measurement of number of alleles (variants). Number of alleles is much more sensitive to population history than heterozygosity. For this reason, it is preferable to heterozygosity for evaluating risks due to reductions in population size and inbreeding.

We calculated the average number of alleles per gene in both populations (Table 2). In spite of the larger sample size in Green Valley, the number of alleles found in Lagunitas/Olema is close to twice that in Green Valley. Strictly speaking, such

comparisons should be weighted by the number of observations used to estimate them. Allelic richness is a comparative measure of the number of alleles, corrected for sample size, when calculated in samples of unequal size. Allelic richness in Lagunitas/Olema is exactly double that in Green Valley (Table 2). Because of the small sample sizes in the other Russian tributaries, calculation of allelic richness there would be inappropriate.

Table 2:

Population	Sample size	Expected Heterozygosity	Observed Heterozygosity	Mean No. Alleles	Allelic Richness
Green Valley	189	0.5541	0.6752	6.17	5.44
Lag/Olema	114	0.6467	0.6429	11.50	10.88
Redwood	1	0.7500	0.7500	1.75	N/A
MarkWest	4	0.5076	0.5490	2.65	N/A

Measures of genetic distance

Fst, or the standardized variance in allele frequencies between populations, is the oldest and one of the most widely used measures of genetic distance between populations. We calculated Fst using the estimator of Weir and Cockerham (1984) in the Genetix (Belkhir et al. 2002) and GenePop (Raymond and Rousset 1995) software packages. The value estimated was 0.130, which can be interpreted as approximately 13% of all variation found was partitioned between populations. This level of differentiation is similar to that found between steelhead trout populations in the Smith (Klamath ESU) and the Santa Ynez (Southern California ESU) Rivers (Garza et al. in prep) and indicates substantial differentiation. It should be noted that the Fstmax<1-He (Hedrick 1999). Since overall He is 0.662, this means that the maximum Fst that is mathematically possible for this comparison is less than 0.338. Another way to look at differentiation is to estimate the mean number of migrants per generation (Nm), which is frequently calculated by transforming Fst. Nm calculated in this way is 1.67. However, this method is subject to the same effect of variability on maximum values, as well as numerous other sources of error. The private alleles method of Slatkin (1985) should perform better with highly variable loci. When using Slatkin's method, as implemented in Genepop (Raymond and Rousset 1995), the estimated number of migrants per generation (Nm) is 0.57. Both of these values are extremely low and indicative of little or no contemporary migration between populations. The Fst and Nm measures are average values calculated across many genes and are meant to measure interactions over many generations. They assume equilibrium in population size and migration rates, which have almost definitely not been constant, and thus the values above should be interpreted with caution. However, individual values can also be informative in evaluating recent gene flow. Two of the 18 genes, Omm1080 and OtsG68, examined in our work have alleles present in approximately 90% of the gene copies in the Russian River that are not present at all in the Lagunitas/Olema system (Figure 1). This observation is particularly important in that the bottleneck in the Russian River would most likely increase its genetic distance from other populations through the loss of some of the alleles present in these closely related populations. However, the presence of many alleles not present in the Lagunitas/Olema system, which has no evidence of a recent bottleneck, suggests that

the distance is not the result of genetic drift removing alleles from an ancestral distribution that was similar in both rivers, but lack of substantial recent gene flow.

Individual-based genetic measures

A key question with respect to any captive breeding project is the relationship between individuals used as mating partners, or the relationship between individuals who are released and those who they will encounter, and possibly mate with, when they're released. Breeding between individuals that are close kin causes inbreeding, which usually leads to a decrease in survival or fecundity known as inbreeding depression, whereas mating between highly distinct individuals leads to outbreeding depression. Factorial correspondence analysis (Smouse and Long 1988) provides a graphical way of summarizing genetic differences between groups of individuals. Similar to principal components analysis, it is a canonical method for displaying joint differences in allele frequencies in individuals and populations. These differences can be represented in 3 dimensional space. The results of such an analysis are shown in Figure 2.

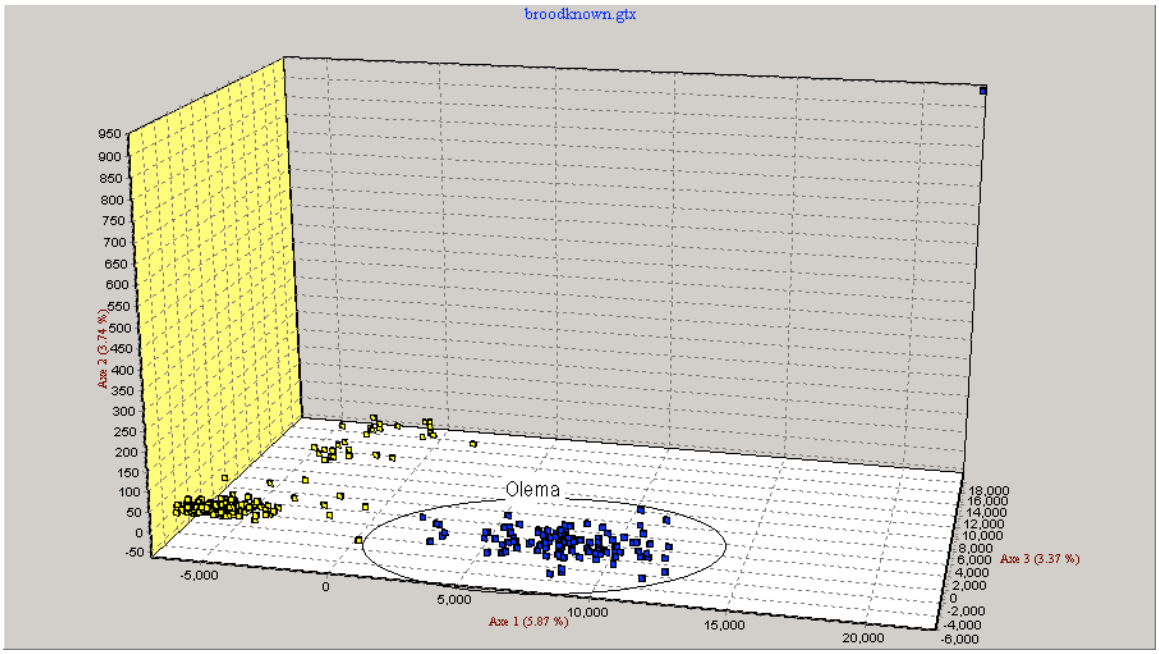

Figure 2: Factorial Correspondence Analysis of 308 coho salmon captured for the Russian River Captive Brood Program. Individuals in yellow are from the Green Valley population, while those in blue are from the Lagunitas/Olema population.

Assignment tests

Genetic assignment tests provide another individual-based method for examining genetic similarity. Assignment analyses examine each individual fish and compares it's genotype with that of a number of potential parental populations. The likelihood of that genotype being from each of the groups is computed and the highest value is the group chosen for assignment. There are many ways to compute and compare these likelihoods, but they usually only differ in assignment results when two different potential parental populations are similar. Since the fish from Mark West and Mayacama aren't numerous enough to derive reasonable estimates of allele frequency, only the Green Valley and

Lagunitas/Olema fish were used as potential populations for assignment. We employed both frequency- and Bayesian-based assignment tests. Both methods gave the same results. The results of such analyses are usually displayed in matrix form. However, the assignment test analyses revealed no cross assignments, that is assignment efficiency was 100%, so no matrix was prepared. The 5 fish from the other Russian River tributaries were used in an assignment test analysis where the likelihood of their origin in either of the two larger populations was compared. In all 5 cases, they were assigned to the Green Valley population with high probability.

Coho Salmon Population Structure

At the time of writing, a final report (Hedgecock et al. 2002) has recently become available describing work on coho salmon genetic population structure in California performed under a contract from the Sonoma County Water Agency to the Bodega Marine Lab. In addition, the Santa Cruz Laboratory (SCL) is in the later stages of a parallel study on coho salmon population structure. These studies differ somewhat in the populations and year-classes included in the study and in the number of genes analyzed. For example, the SCL study estimates the frequency of 401 alleles in their analyses whereas the Bodega study estimated the frequency of 111 alleles. The accuracy and precision of almost all population genetic analyses, including gene tree topology and branch lengths, increase with the size of the dataset (Nei and Kumar 2000). A phylogeographic tree summarizing the preliminary results of the SCL population structure work is found in Figure 3. The Bodega study has largely concordant results on population structure.

The tree in Figure 3 indicates that there are no coho salmon populations, among those sampled, which are closely related to the Russian population and therefore a good candidate for supplementation in Russian River recovery efforts. An additional result of interest is that the population in Lagunitas/Olema appears to have been influenced by outplanting from the Noyo River. In contrast, the Bodega study found that the Noyo River-derived stock used in production efforts at WSH in the early 1990s bears no resemblance to the coho salmon currently found in the Russian River.

Breeding matrix

We used the genetic data described above to provide a matrix for breeding of coho salmon from the Russian River collected for year 1 of the recovery program. The basis for the breeding matrix is a measure called the coefficient of relatedness (Rxy). This value is the probability that an allele is identical by descent (Queller and Goodnight 1989) and Rxy values are thus highly correlated with the genetic relationships expected between relatives. The asynchronous nature of reproductive maturation in the species necessitates an adaptive approach to such a breeding matrix. Since it is not known ahead of time which males will be producing milt when any given female is producing eggs, it is not sufficient to simply determine which males and females are optimal mating partners. Instead, we treat each female as a focal individual and provide a rank order for every male. The matrix then describes which matings are more preferable than others and allows all available males to be evaluated (ranked) relative to one another when a female is producing eggs. In addition, in a captive population where many of the individuals are closely related, there will be many close kin that should not be mated together and,

therefore, many crosses which should not be performed at all. We found that a large proportion of all comparisons of individuals from the Russian River population were between close relatives, whereas those between individuals from Lagunitas/Olema were not (Figure 4). We set the threshold above which individuals should not be mated together at $Rxy \geq 0.25$, which corresponds to the level of relatedness characteristic of half siblings. We chose this threshold because it represents a reasonable compromise between avoiding inbreeding and ensuring that each female has partners available. Moreover, the next biologically meaningful threshold corresponds to the level of relatedness characteristic of first cousins, which are known to mate frequently in natural populations with inbreeding avoidance mechanisms. As discussed above, because of the large genetic distance between fish from the Lagunitas/Olema and Russian systems and the similarity between the fish from the different Russian River tributaries, we include all Russian River fish in this matrix, but those from Lagunitas/Olema are excluded. Gender assignments are based on the results of ultrasound analyses (Conrad and Arkush, pers. comm.). We treated individuals that were either reproductively immature or not found during the ultrasound analyses as potentially either male or female. The matrix is found in Appendix 1.

Conclusions and Management Recommendations

Our data indicate that the Russian River and Lagunitas/Olema Creek populations of coho salmon constitute two separate populations, with little or no contemporary gene flow between them. Because at least some of the differences between these stocks are likely involved in local adaptation, interbreeding the two stocks could cause significant outbreeding depression and is not recommended. However, fish from the 3 tributaries of the Russian River in the 2001 year class can be treated as one population and interbred.

Outbreeding depression occurs when individuals that come from populations that are genetically distinct and adapted to different conditions are mated together, resulting in offspring that are well-adapted to neither set of conditions and thus have reduced fecundity and/or survival. While this occurs under natural conditions, when mechanisms that would prevent such matings are circumvented in a captive situation, it can contribute to continuing population decline and propel populations into the extinction vortex (Gilpin and Soulé 1986). It is very difficult to know *a priori*, what level of molecular genetic divergence between uniting gametes is sufficient to cause outbreeding depression. However, the high levels of adaptation generally found in salmonids, and the large genetic distances in central California coho salmon (Weitkamp et al. 1995; Hedgecock et al. 2002; Garza and Gilbert-Horvath, unpublished data), indicate that the risk of disrupting coadapted gene complexes through human-mediated hybridization is high. The observation that generations of supplementation of coho salmon in the Russian River with out-of-basin stocks has failed to produce a naturally spawning population is further evidence that local adaptation is poorly understood and potentially strong in central California. Given these considerations, interbreeding genetically distinct stocks runs a high risk of producing maladapted offspring that will not be able to successfully survive and reproduce, and is strongly discouraged.

Another area of concern when recovery efforts involve captive breeding is inbreeding and inbreeding depression. Inbreeding occurs when closely related individuals produce

offspring. The effects of inbreeding and the importance of genetic variation in the continuing persistence of endangered species, including salmonids (Wang et al. 2002), are well documented. A breeding strategy that simultaneously maximizes the effective population size in the resultant offspring and eliminates or reduces matings between kin is easily obtainable with genetic data and will achieve both of the above-mentioned goals. However, because of the lack of complete gender information and the asynchrony in reproductive maturation, the construction of a specific breeding matrix is necessarily adaptive and "last minute". We have used pairwise estimates of kin relatedness for all individuals, and gender information inferred from ultrasound, to produce such a matrix. This will allow hatchery staff to evaluate the selection of males available when a focal female becomes reproductively mature and select matings that will result in maximum effective size and minimum inbreeding. Some potential matings should not be performed, due to close kinship, and are well indicated. Although perhaps counterintuitive, if space limitations are an issue, it can be better not to breed a female at all then to cross a pair that will produce highly inbred offspring.

Because of the limited genetic variation, due to a very limited number of spawning adults that has given rise to the broodstock, the Russian River fish run the risk of inbreeding depression even with optimal mating protocols. There are several potential strategies to address this concern. One is to move gametes across year classes, either through cryopreservation of sperm, or through rearing strategies that either accelerate or retard reproductive maturity. A preliminary comparison of the 2001 and 2002 Russian year classes has shown that they are more similar to one another than to any other examined stock (Figure 3), and that they are also sufficiently dissimilar that crossing different year classes would decrease inbreeding and increase genetic variation for the focal year class. Moreover, it is known that both of these groups have the necessary adaptations to successfully breed in the Russian River. We have selected a set of 25 presumptive males that would be good candidates for sperm cryopreservation and would be representative of genetic diversity in the 2001 brood year to facilitate this strategy. It should be noted that SCL scientists have recently documented 2-year-old immature coho salmon in Scott Creek (Santa Cruz Co.; S. Hayes, pers. comm.), which indicates that there is some natural gene flow across brood years in California.

Another strategy for increasing genetic diversity and minimizing inbreeding is to introduce genes from another stock, as has been done for Florida panthers and other species. However, this strategy is risky for a species with the level of local adaptation found in salmon. Thus no discussion of such a strategy should occur until after a careful experimental evaluation of fitness in fish which result from such a cross. This would include captive crosses, fitness measurement in captivity and carefully controlled and monitored release and recapture in a site that would provide little opportunity for experimental fish to interact with other extant populations. A possible example of such a site might be Salmon Creek in southern Sonoma County, but should not be in the Russian River. Moreover, since many effects of outbreeding depression do not become apparent until the second generation of crossing, due to first generation heterosis (hybrid vigor), non-experimental releases of crossed fish should not be considered before evaluating fitness in two offspring generations. Since inbreeding effects are generally mild in the short term, this would be a reasonable time frame in which to address the risk posed by inbreeding in this population and might be considered as a long-term solution to the

potential problem of inbreeding. While it is prudent to be concerned about potential inbreeding, with current population numbers as low as they are, demographic factors are much more likely to result in extinction than genetic factors (Lande 1988) and the initial focus of the program should be to increase population numbers.

The lack of knowledge about coho salmon population relationships at the outset of this program led to adoption of a precautionary strategy that included capture of fish from Lagunitas/Olema as a potential donor stock for supplementation in the Russian River. The extremely small population sizes in the Russian was expected to give rise to excessive coancestry between Green Valley coho salmon and this has turned out to be the case. However, the revelation that the two populations are extremely divergent and inappropriate for hybridization, has led to a dilemma about what to do with the Lagunitas/Olema fish currently held at WSH. At present, Marin County fish from all three broodyears are being held at WSH and the 2001 year class will become reproductively mature this winter.

There are several broad options for what to do with these fish. The first is to simply let the fish die in the hatchery. The second is to release them into their stream of origin. The third is to release them in some other stream. It would also be possible to breed them in the hatchery and then release the offspring, either in the stream of origin of their parents or in some other stream. For the reasons mentioned above, we will not consider hybridizing the two populations, outside of the experimental strategy outlined above.

The first strategy is perhaps the simplest. However, the WSH facility is space-limited and experience with raising coho salmon in Santa Cruz and Washington State has shown that, in captivity, some fish never become reproductively mature and die at four years of age. Because there is not sufficient space at WSH, letting these fish die naturally would not be an option and they would need to be killed. Political opposition to such a strategy has been fierce in other captive rearing programs with ESA listed species.

The next two options involve planting these fish into a freshwater stream. The obvious choice is to return them to the Lagunitas/Olema system. However, this is not an optimal choice for two reasons. First, a promise was made to members of the west Marin County community that fish taken from the Lagunitas/Olema system would not be returned there. Second, there are several potential risks to such a reintroduction. It is possible that the fish might transmit some disease present in the Russian River to Lagunitas/Olema Creek. In our opinion, this risk is remote, given the proximity of the two watersheds and the veterinary precautions taken at WSH. Second, if the fish captured came from a limited number of family groups and a large number of individuals released were successful in reproducing, then the variance in family size in the watershed would possibly be increased, which would lead to a decrease in effective population size (Ryman and Laikre 1991). However, the genetic data indicate that the fish held at WSH are outbred and are not from a limited number of families (Figure 4). Moreover, it is unclear whether fish held throughout their life cycle would reproduce successfully at the same rate as those that have undergone a sea migration.

A promising option is the release of the Lagunitas/Olema fish in a stream where such risks do not exist. Since many of the streams in Central California currently do not have populations of coho salmon, but have recently lost them due to extinction, this strategy would both resolve the problem of what to do with the fish currently being held at WSH and help to restore recently extinct populations. This strategy has been endorsed in the

State Southern Coho Restoration Plan (CDFG 1998) and is planned for streams in Santa Cruz and San Mateo counties where coho salmon have recently gone extinct. An additional benefit of such a reintroduction is that it would provide a hedge against extinction of the Lagunitas/Olema population in the event of a catastrophe such as a disease epidemic or a toxic spill. The establishment and maintenance of more than one population for threatened and endangered species (and other distinct population segments) is a central goal in many recovery plans. One specific question raised by such a reintroduction is which watershed would be the focus of releases. Both geography and the genetic data provide some guidance on this issue. First, because of the geographically specific nature of genetic variation in coho salmon, and salmonids in general, a reintroduced population should be close to the original source. This way, any straying (migration) would not have a high probability of increasing gene flow between genetically distinct lineages. Second, genetic data for both coho salmon (Figure 3) and steelhead trout (Garza et al. in prep), indicate that there is reduced gene flow between the Russian and streams to the south. This is likely due to the ocean circulation patterns associated with Bodega Head, which leads to distinct oceanographic patterns to the north and south (B. MacFarlane, pers. comm.). Taken together, these observations suggest that a good release site would be south of Bodega Head. The only sizable watersheds between Bodega and Lagunitas/Olema are Walker Creek and the Esteros. Walker Creek is especially attractive because of its size, the presence of good salmon spawning habitat and landowner support. For example, the Walker Creek Ranch is supportive of a proposal to reintroduce coho salmon on their property and have indicated their desire to create an education program that would focus on the salmon and, at least partially, provide follow-up and monitoring. Such a release program might then have educational benefits in addition to the primary goals of the release. In summary, a release of fish into Walker Creek would resolve the dilemma, due to the adaptive nature of the recovery program, of what to do with the of the Marin coho held at WSH, would potentially establish a satellite population to Lagunitas/Olema that would provide a buffer against catastrophic events and would provide educational opportunities to Marin County children.

Such a release would also have some risks associated. These are of two types. First and foremost, it is not at all clear that such a reintroduction would result in the successful establishment of a spawning population, or even result in any spawning at all. However, it should be pointed out that there is no chance that these fish will pass on their genes if they are left in the hatchery to die, so any spawning that occurred would be a net gain. Second, there is a chance that some fish released in Walker Creek might stray into Lagunitas/Olema and cause harm. However, the chance of either of these things occurring, especially that of causing harm, is unlikely and the chance that they would both occur is vanishing small, or effectively zero. This is because fish released in Walker Creek as adults will almost definitely not stray elsewhere, even if they imprinted on Lagunitas/Olema as juveniles. There are several reasons for this. First, the way that coho salmon homing works is that, while on migratory pathways in the ocean, they "smell" their streams due to outflow into the ocean. They will not have that opportunity in Walker Creek. Second, to our knowledge there are no documented cases of coho salmon entering freshwater and then returning to salt water.

Importantly, even if some fish did stray into Lagunitas/Olema there is little to no chance that they would cause harm to that population. These fish are the same ones taken

from Lagunitas/Olema and they are native there. There has not been "damage", genetic or otherwise, from the 2 years of residence at WSH. The only difference between these fish and the adults that will return to Lagunitas/Olema is that they have not been subject to the same natural selection during the ocean phase of their life history and they have been exposed to a different water source for several years. The risks of disease transmission are remote and, to our knowledge, there are no documented cases of such transmission due to movement of salmonids, in spite of massive out of basin transfers of salmon and trout in California during the last century. The effects of a few fish straying from Walker Creek into Lagunitas/Olema would have no negative effect on effective population size both because they are not inbred and because, even if a large percentage of them migrated and successfully spawned, they would constitute a relatively small percentage of all spawners (Ryman and Laikre 1991). Over the long term, the presence of such a satellite population would most likely be beneficial to the Lagunitas/Olema population both by providing a hedge against extinction in the face of a catastrophic event and because the relatively different evolutionary forces experienced by the two populations of the same ancestry will provide greater genetic, and perhaps physiological, diversity for this stock to adapt to future environmental or ecological changes.

Finally, it is important to note that our results and interpretations are based on the analysis of the genetics of one year class of coho salmon. Because of the relatively rigid three year life cycle of coho salmon in California, it is necessary to evaluate the generality of these results to the two other year classes of these stocks. Preliminary results (see Figure 3) indicate that the genetic distances of the 2002 year class are similar to those of the 2001 fish. A report on the genetics of the fish captured for the second year of this program will be provided in 2004.

Acknowledgements

We thank Drs. Devon Pearse, Sean Hayes, Bruce MacFarlane (SCL) and Dr. Kristen Arkush (BML) for insightful discussion, comments and observations. In addition, Dr. Pearse assisted with construction of the breeding matrix and Mari Rosales assisted in data collection. Louise Conrad (CDFG) provided the gender assignments based on ultrasound results collected by her, Brett Wilson (CDFG) and Dr. Arkush. This work was funded by the NOAA Fisheries Southwest Region and Southwest Fisheries Science Center operating funds. The second year of genetic analysis is funded by a contract from the Pacific States Marine Fisheries Commission that was facilitated by Bob Coey (CDFG).

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Allele Frequencies for Two Genes in coho Salmon Held at Warm Springs Hatchery

Allele Size	Allele Frequency		Allele Size	Allele Frequency	
Gene OtsG68	Lag/ Olema	Green Valley	Gene Omy1080	Lag/ Olema	Green Valley
191		16.18	233	0.48	
203	6.88		283		23.63
211	6.42		289		0.55
219	0.46		291		5.22
223	6.42		299	11.43	
231	1.38		309	7.62	
239		10.29	313	0.95	
247	0.92	4.41	325	9.05	
275	1.83	0.88	329	4.29	
295	2.29	0.88	333	1.90	
299		20.29	337	1.43	
303	3.21		341	5.24	
307	18.81	4.12	345	0.95	
311	13.76		349	4.76	
315	6.42		353	7.14	1.65
319	5.05		357	1.90	
327		0.29	361	4.76	10.99
331	0.92	0.29	365		43.68
335	0.92	0.29	372	5.24	
339		42.06	373	9.52	
343	18.81		376	2.38	
347	0.46		377	12.38	
351	1.83		385	0.48	
355	2.75		393	4.29	
360	0.46		401	2.86	
			405	0.95	
			457		14.29

Figure 1

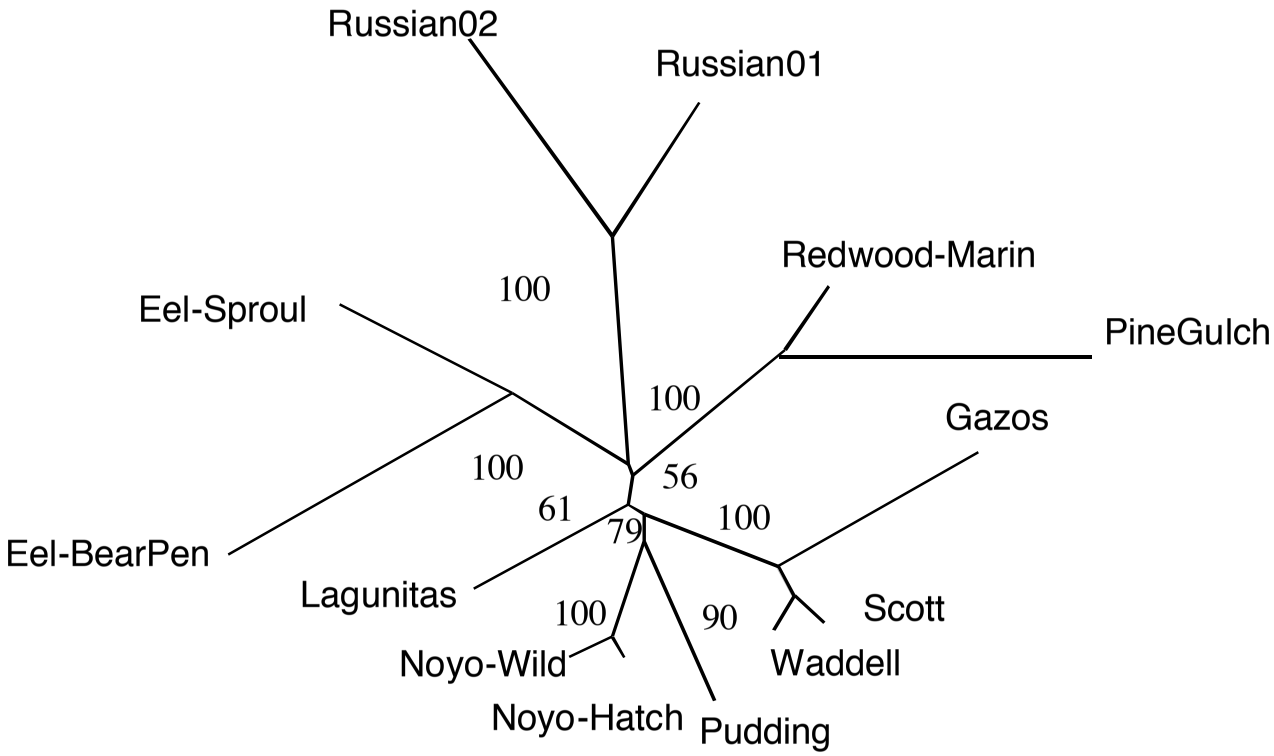

Figure 3: Neighbor-joining on Cavalli-Sforza/Edwards Chord Distances Bootstrap values for 10K resampled datasets. 401 alleles used in analyses.

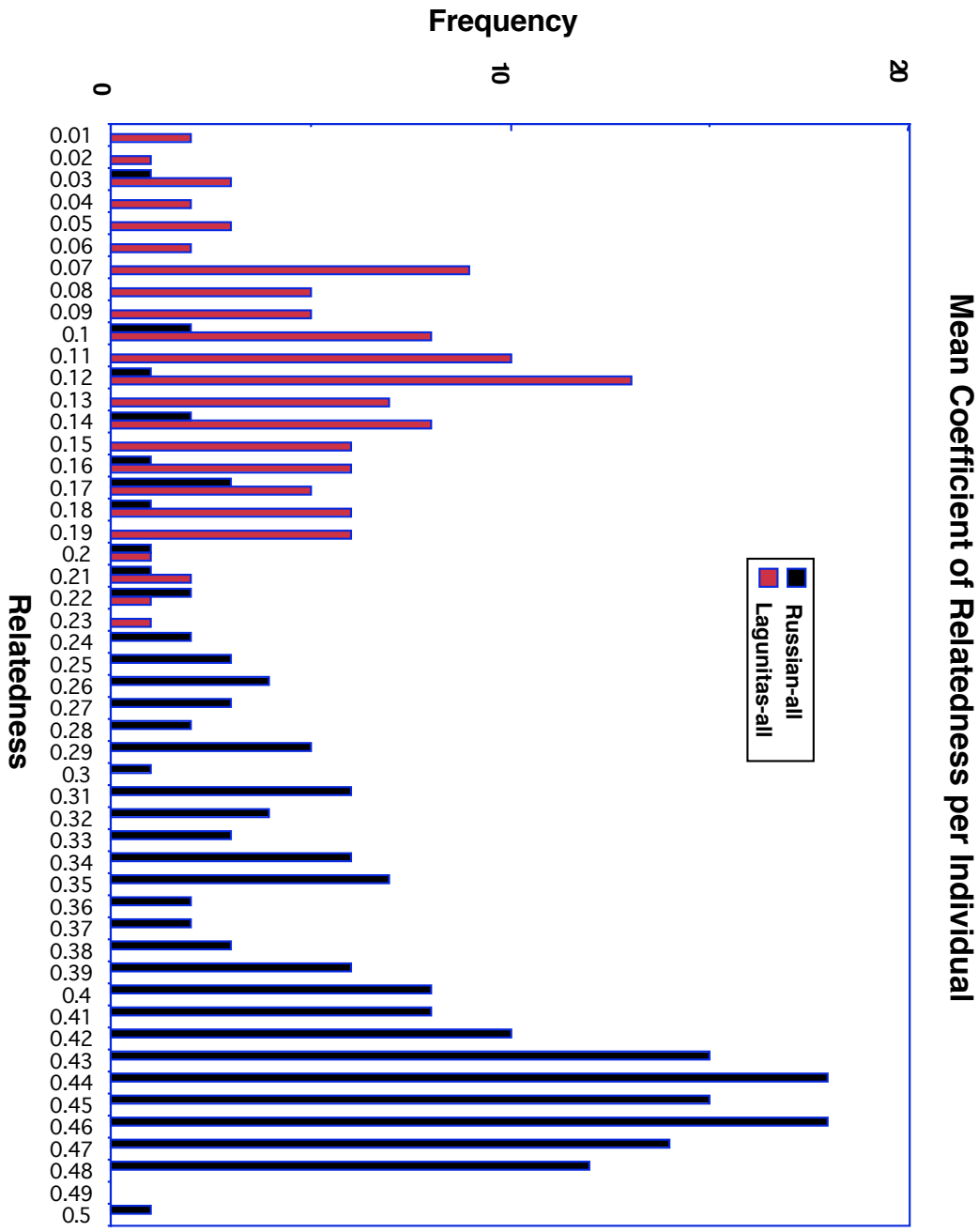

Figure 4: Distribution of mean Rxy value per individual in the Russian and Lagunitas/Olema populations.